

ON-LINE SUPPLEMENTAL DATA

Utility of serum non-esterified fatty acids as dietary biomarkers of habitual fat intake in women

Sandi M. Azab,^{1,2} Russell J. de Souza,^{3,4} Koon K. Teo (for the FAMILY investigators),³ Sonia S. Anand,^{3,4} Natalie C. Williams,³ Jordan Holzschuher,¹ Chris McGlory,⁵ Stuart M. Philips⁵ and Philip Britz-McKibbin¹

¹ *Department of Chemistry and Chemical Biology, McMaster University, Hamilton, ON, Canada*

² *Department of Pharmacognosy, Alexandria University, Alexandria, Egypt*

³ *Department of Medicine, McMaster University, Hamilton, ON, Canada*

⁴ *Department of Health Research Methods, Evidence, and Impact, McMaster University, Hamilton, ON, Canada*

⁵ *Department of Kinesiology, McMaster University, Hamilton, ON, Canada*

Address Correspondence to Philip Britz-McKibbin, Department of Chemistry and Chemical Biology, McMaster University, 1280 Main St. W. Hamilton, ON, Canada, L8S 4M1.

Tel: +1-905-525-9140 x22771.

E-mail: britz@mcmaster.ca

Supplemental Figure S1, Table S1, Table S2, Table S3, and Table S4

Supplemental Figure.

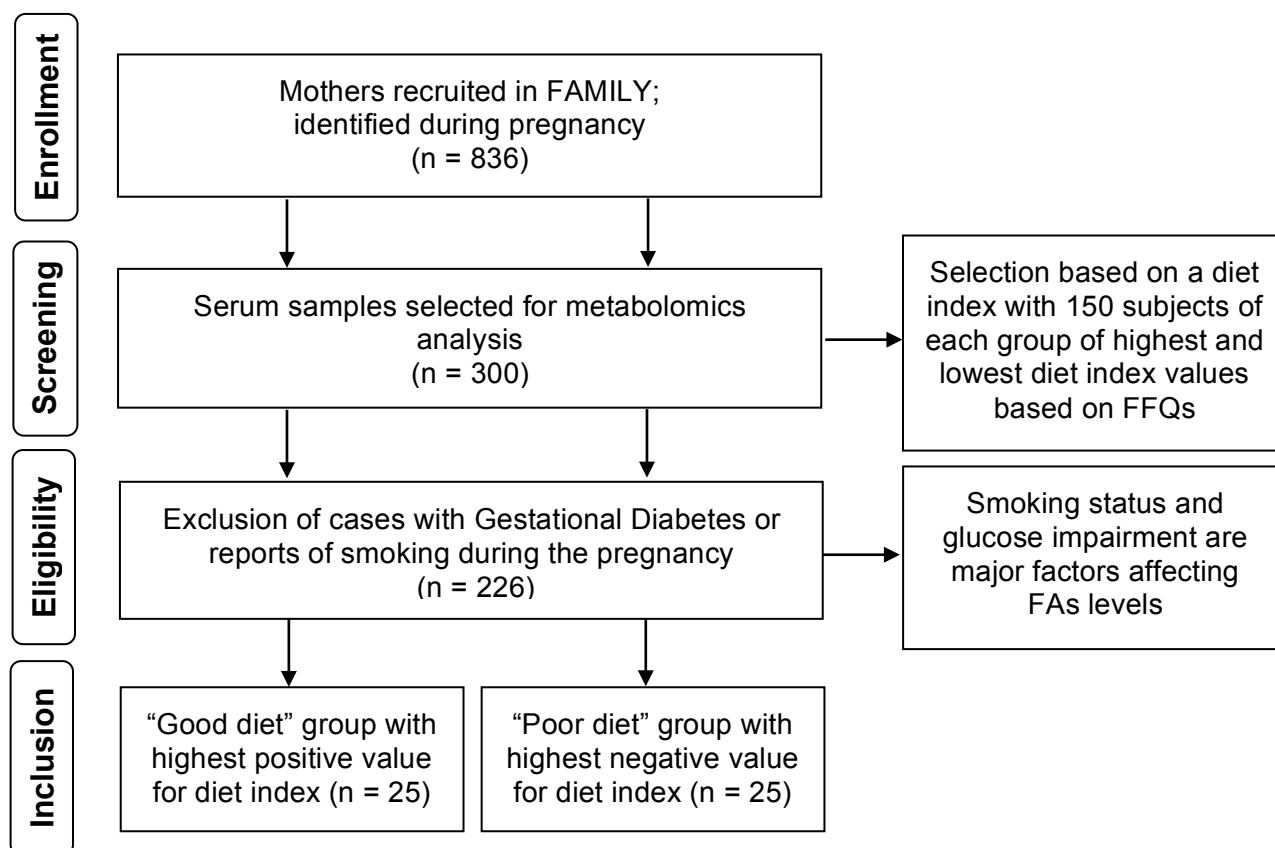


Figure S1. A flow diagram outlining selection criteria used in a cross-sectional study involving participants from the FAMILY birth cohort ($n=50$) having contrasting eating patterns as measured by a diet quality index score. NEFA and total hydrolyzed fatty acids analysis was performed using MSI-NACE-MS on maternal serum samples collected during the second trimester of pregnancy.

Supplemental Tables

Table S1. Serum fatty acids (NEFA and/or total FA) measured by MSI-NACE-MS from FAMILY cohort ($n=50$).

	Fatty Acid	Molecular Formula	m/z^a	RMT ^b	Mass Error (ppm)	%RSD ($n = 8$) ^c	Ratio of NEFA/Total (%) ^d
1	Pelargonic acid (9:0)*	C ₉ H ₁₈ O ₂	157.123	1.214	0.7	9.8	-
2	Capric acid (10:0)*	C ₁₀ H ₂₀ O ₂	171.139	1.123	2.6	22.6	-
3	Lauric acid (12:0)	C ₁₂ H ₂₄ O ₂	199.170	1.085	2.0	14.1	137 ± 80
4	Myristic acid (14:0)	C ₁₄ H ₂₈ O ₂	227.202	1.054	3.5	8.6	85 ± 42
5	Pentadecanoic acid (15:0)	C ₁₅ H ₃₀ O ₂	241.217	1.041	2.1	11.0	34 ± 18
6	Palmitic acid (16:0)	C ₁₆ H ₃₂ O ₂	255.233	1.028	1.2	12.6	19 ± 9
7	Heptadecanoic acid (17:0)	C ₁₇ H ₃₄ O ₂	269.249	1.017	2.2	13.2	28 ± 14
8	Stearic acid (18:0)	C ₁₈ H ₃₆ O ₂	283.264	1.005	4.6	13.6	22 ± 13
9	Arachidic acid (20:0)*	C ₂₀ H ₄₀ O ₂	311.296	0.983	3.8	13.6	-
10	Behenic acid (22:0)**	C ₂₂ H ₄₄ O ₂	339.327	1.017	0.6	15.9	-
11	Myristelaidic acid (14:1)	C ₁₄ H ₂₆ O ₂	225.186	1.061	1.8	13.1	42 ± 13
12	Palmitoleic acid (16:1)	C ₁₆ H ₃₀ O ₂	253.217	1.035	2.4	8.5	85 ± 45
13	Heptadecenoic acid (17:1)	C ₁₇ H ₃₂ O ₂	267.233	1.024	1.8	7.9	120 ± 50
14	Oleic acid (18:1) ^x	C ₁₈ H ₃₄ O ₂	281.249	1.012	1.1	12.7	69 ± 33
15	Gondoic acid (20:1)	C ₂₀ H ₃₈ O ₂	309.280	0.99	4.2	13.1	87 ± 50
16	Nervonic acid (24:1)**	C ₂₄ H ₄₆ O ₂	365.342	0.946	5.2	15.5	-
18	Linoleic acid (18:2) ^x	C ₁₈ H ₃₂ O ₂	279.233	1.018	0.4	7.7	18 ± 9
17	Eicosadienoic acid (20:2)	C ₂₀ H ₃₆ O ₂	307.264	0.995	3.1	11.8	33 ± 30
19	Linolenic acid (18:3) ^x	C ₁₈ H ₃₀ O ₂	277.217	1.021	1.4	8.1	71 ± 32
20	Dihomo-γ-linolenic acid (20:3 n -6)	C ₂₀ H ₃₄ O ₂	305.249	1.004	2.3	10.6	-
21	Arachidonic acid (20:4 n -6)	C ₂₀ H ₃₂ O ₂	303.233	1.025	2.3	5.1	5 ± 4
22	Eicosapentaenoic acid (20:5 n -3)	C ₂₀ H ₃₀ O ₂	301.217	1.027	5.0	9.6	5 ± 4
23	Adrenic acid (22:4 n -6)**	C ₂₂ H ₃₆ O ₂	331.264	0.991	4.5	8.5	-
24	Docosahexaenoic acid (22:6 n -3)	C ₂₂ H ₃₂ O ₂	327.233	1.029	2.4	9.0	6 ± 6

^a Accurate mass of fatty acid detected as its intact molecular ion $[M-H]^-$ under negative mode ionization.

^b Migration time of fatty acids relative to the internal standard, C18d35 for data normalization.

^c Relative standard deviation of repeated QC samples introduced in each run for assessment of technical precision.

^d Relative abundance of NEFA as compared to total (hydrolyzed) FA based on a ratio of their relative peak areas.

*Fatty acid only detected in serum NEFA analysis

**Fatty acid only detected in serum for total fatty acid analysis

^x Isomeric resolution of cis and trans geometrical isomers for fatty acids were not achieved.

Table S2. Multiple linear regression model to predict levels of ω -3 fatty acids (dependent variable) as a function of the diet quality index score with adjustments for covariates, BMI, cholesterol, and HDL.

Independent variables	Coefficient	<i>p</i>-value	<i>r</i>
<i>EPA-Total</i>			
Diet index (DI)	0.018	0.0020	0.44
DI, BMI	0.017	0.0030	0.44
DI, BMI, cholesterol	0.013	0.029	0.52
DI, BMI, cholesterol, HDL	0.006	0.19	0.75
<i>DHA-Total</i>			
Diet index (DI)	0.015	0.0030	0.42
DI, BMI	0.015	0.0030	0.42
DI, BMI, cholesterol	0.009	0.061	0.57
DI, BMI, cholesterol, HDL	0.008	0.10	0.58
<i>EPA-NEFA</i>			
Diet index (DI)	0.012	0.017	0.34
DI, BMI	0.012	0.025	0.35
DI, BMI, cholesterol	0.013	0.019	0.37
DI, BMI, cholesterol, HDL	0.009	0.087	0.52
<i>DHA-NEFA</i>			
Diet index (DI)	0.013	0.017	0.34
DI, BMI	0.013	0.027	0.35
DI, BMI, cholesterol	0.014	0.024	0.36
DI, BMI, cholesterol, HDL	0.011	0.081	0.45

Table S3. Multiple linear regression model to predict levels of ω -3 PUFA (dependent variable) as a function of total ω -3 daily servings with adjustment for covariates, BMI, cholesterol, and HDL.

Independent variables	Coefficient	<i>p</i>-value	<i>r</i>
<i>EPA-Total</i>			
T ω -3	0.373	0.020	0.34
T ω -3, BMI	0.363	0.024	0.35
T ω -3, BMI, HDL	0.295	0.055	0.50
T ω -3, BMI, cholesterol, HDL	0.209	0.076	0.76
<i>DHA-Total</i>			
T ω -3	0.520	1.9×10^{-6}	0.53
T ω -3, BMI	0.518	2.7×10^{-6}	0.53
T ω -3, BMI, HDL	0.446	4.7×10^{-6}	0.68
T ω -3, BMI, cholesterol, HDL	0.434	8.5×10^{-6}	0.69
<i>EPA-NEFA</i>			
T ω -3	0.329	0.024	0.32
T ω -3, BMI	0.320	0.029	0.34
T ω -3, BMI, HDL	0.337	0.025	0.36
T ω -3, BMI, cholesterol, HDL	0.283	0.039	0.54
<i>DHA-NEFA</i>			
T ω -3	0.308	0.056	0.27
T ω -3, BMI	0.297	0.067	0.30
T ω -3, BMI, HDL	0.310	0.061	0.32
T ω -3, BMI, cholesterol, HDL	0.265	0.095	0.44

Table S4. Spearman correlation coefficients between circulating DHA and EPA (molar concentrations) measured as their NEFA and hydrolyzed (total) serum ($n = 48$) in pregnant women ($n = 50$) from the FAMILY study.

	Serum Total			
	DHA (22:6n-3)		EPA (20:5n-3)	
Serum NEFA	<i>r</i>	<i>p-value</i>	<i>r</i>	<i>p-value</i>
EPA (20:5n-3)	0.44**	0.0020	0.57**	0.000020**
DHA(22:6n-3)	0.29*	0.049	0.44**	0.0020**

*Correlation is significant at the * 0.05 level (2-tailed) ** 0.01 level (2-tailed)*